"The contributed chapters in the book written by the faculties of science stream in the light of the recent thinking and developments in the field of science and education.- Science& Technology is now dominates almost every field of our activities.In summary, The faculties (Science stream) of GEMS Arts & Science college have made an excellent attempt to bring about this book Homo Scientia", covering almost all the important areas from biological sciences to artificial intelligence. Every article has its own merits in both academic and research fronts..I record my grateful appreciation and thanks to the contributors of this book for their untiring efforts."

Dr. Balagopalan Unni

Gems Arts & Science College (Affiliated to University of Calicut),Ramapuram, kadungapuram (PO), Malappuram (DT) Pin -679321

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HOMO SCIENTIA

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Dr. K Gopalakrishnan,
Smitha Pramod V

GEMS

HOMO SCIENTIA

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Brief Biography

Dr. B.G.Unni, (Balagopalan Unni) Ph.D
(Allahabad central University)
FRES (London), FIANSc , FISAgBc, FICCE

Former Chief Scientist and Area Coordinator (Biotechnology & Biological Sciences) DADD and Fulbright Fellow retired from CSIR service in 2015 after 38 years of research career at CSIR North East Institute of Science & Technology Jorhat Assam. Appointed at Assam down town University as Director-Research in March 2015 and continued up to June 2019 and then re-designated as Adviser Research in August 2019). Back in Kerala, Dr.Unni is appointed as Director Academic & Research at GEMS College of Arts & Science affiliated to University of Calicut from August 2019. Both the positions are on honorary basis to strengthen the institutions in research areas. He did his BSc Biology (1972-74, Ewing Christian College, Alld University), MSc in Biochemistry(1974-76)(Second Rank) and Ph.D in Biochemistry from Allahabad University(1976-80) and PDF in Molecular Biology from Texas A&M University, USA(1988-91). Dr. Unni is specialized in Biochemistry, Molecular Biology, and Biotechnology and well established in his area of research and completed more than 40 years of research in both basic and applied fields of research. Dr.Unni got more than 130 research papers, 190 abstracts, 35 papers in proceedings, 7 patents,1 technology.18 chapters in books, edited 3 books and 29 students

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received PhD degrees under his guidance and supervision. Dr. Unni had completed more than 20 projects sponsored by Commonwealth Science Council, London, Ministry of Non conventional Energy Sources, Department of Non conventional Energy Sources Govt of India, North Eastern Council Govt of India, Department of Science &Technology, Department of Biotechnology, Central Silk Board,GB Pant Institute of Himalayan Environment and Development, CSIR and DRDO, Ministry of Defense, Govt of India during his scientific tenure at CSIR NEIST. Dr Unni received- Fulbright Travel Award/ Fellowship (USA) Dr. B.M. Das Memorial Science award, Hebrew University Award , H.R. Cama Memorial Travel Award,COSTED Travel Award,. DAAD- fellowship-Germany, Well Mark International Scholarship (USA) & Technology award in life sciences by CSIR,Govt of India . Best Fulbright Alumni Chapter Leader-South Asia Selected by the United States Education Foundation In India (USIEF), New Delhi .Nominated to represent India at the International Fulbright Scholars meet at Marrakech, Morocco- Nominated by United States Education Foundation In India, New Delhi . Dr. Unni is in the editorial board of more than eight indexed journal in the country .Dr.Unni was nominated to various state and central committees such as High power committee for development of sericulture activities Muga, Eri,Tassar and Mulberry in Assam nominated by Governor of Assam, .Expert in the area of non mulberry sericulture, Ministry of Textiles, Advisory Board, Post graduate Biotechnology programme, Academic Council, Assam Agricultural University, Research Council, Central Silk Board, Ministry of Textiles , DBT's Nominee for Biosafety Committee ,Vice President SBC (India) Indian Institute of Science Bangalore, Vice President Indian Academy of Neuro-sciences, Member Fulbright Academy of Science & Technology, USA, Board of studies- Botany Nagaland University and Biotechnology Saugar University Madhya Pradesh., Fellow, Indian Academy of Neurosciences & Indian Society of Agricultural Biochemists, Fellow Royal Entomological Society, London UK and Scientific

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Advisor International Foundation of Science, Sweden, Member, Board of Studies Raiganj University (2017----), Member Research Review committee Tea Board of India (2016-2019), Member Advisory Committee Cancer Research Advisory Board, North East Cancer Hospital & Research Institute (2017--) President, Tea Improvement Consortium, Ltd, Tocklai Assam (2018-2020) .

Dr.Unni visited USA, Germany, Israel, Jordan, France, Morocco ,UK, Thailand ,Jordan, Singapore , China and UAE under various exchange program.

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Preface

I am very happy to learn that, the GEMS Arts & Science College is bringing out a series of books written by the faculty in this academic year. The college is occupying a very important position among the colleges in Kerala, the same way the college is having unique standing in both academic and research fronts too. This is because of the excellent management, faculties and the best performances of the students.. I have full confident that in the course of time, and with the sincere commitment and dedication of the faculties , students and with management , the college will attain high level perfection and excellence and became a model college in the state of Kerala

This book entitled " Homo Scientia" had comprehensive research topics in various aspects in the topics of cyber security, biotechnology, microbiology and geology.A brief description about the cybersecurity, the protection of computer set up such as hardware, software data from several threats have been described in the chapter The best practices for deploying and managing IPS network security tools have been explored. The integration of intrusion prevention system (IPS) solutions, adherence to security policies, regular updates, monitoring and the implementation of incident response procedures are considered to be the essential components of a comprehensive network security framework. The risk management in cyber security, various cyber-attack kinds, malware, and some strategies to tackle these attacks are also explained by the authors. A comprehensive overview of the evolution of computer graphics, exploring the advancements in hardware, software, algorithms, and techniques that have propelled the field from its early pixel-based beginnings to the current state of realism etc also described. Optical character recognition has been extensively investigated in the past few years, and has been proven that high recognition rates can be achieved in specific

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application scenarios using some standard and well-studied methods such as neural network, support vector machine (SVM), etc. The possibility of learning an appropriate set of features for designing optical character recognition (OCR) has been investigated

Biotechnology is an interdisciplinary science using modern technologies to construct biological processes in research, agriculture, formulation of pharmaceutical products and other related fields. The better understanding of advances in plant genetic resources, genome modifications, omics technologies to generate new solutions for food security under changing environmental scenarios etc have been discussed in this chapter. The increasing demand for food had a great impact on the agriculture sector to address the various challenges associated with crop productivity. The tremendous advancement in plant research helps in understanding plant biology for sustainable food security, functional ecosystems, crop improvement and human health. One of the sustainable farming techniques is the use of fertilizer at nano level. Nanomaterials that enhance plant nutrition could be considered as an alternative to the conventional chemical fertilizers. one chapter covered the importance of nano fertilizer to enhance metabolic processes in plants and reviewed the concerns in developing nanotechnological methods in the future. Metabolomics has now emerged as a powerful tool for the comprehensive analysis of metabolites within biological systems. One of the chapters provides a review on metabolomics, encompassing its methodologies, applications, potential impact on personalized medicine ,and discusses further the need for advancements in analytical technologies. The antifungal activity of mangroves, particularly Rhizophora species are one of the main sources for fungicidal compounds due to the presence of high concentration of phenols. The antifungal activity of Rhizophora species has been elucidated, and could be further utilized as biocontrol agents for fungal disease in agricultural crops. One of the chapters discussed the species identification and its impact on economical and ecological level in the species like Nutmeg, one of the important medicinal plants that had a greater attention ,however, it was very difficult to differentiate the sexual identity

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in the seedling stages. But the protein content screening among the studied plantlets had differentiated the sexes in the species as explained by the author.

AI (Artificial Intelligence) or machine intelligence enables farmers to enhance the quality and ensure a quick go-to market strategy for crops, and adoption of these algorithms to improve food industries. Artificial intelligence (AI) has also the potential to revolutionize education, from personalized learning to assessment and grading. Additionally, AI-powered tools can provide greater accessibility to students with disabilities, while also enabling more engaging and interactive content. AI continues to develop and become more prevalent in education, towards responsible and equitable implementation. However the negative and positive part of the AI may also be looked into.

The chapters related to microbiological aspects have also been incorporated in this book . Carbapenem-resistant A. baumannii (CRAb), bacteria that cause multi-infections in humans and resistant to multiple drugs too. The study attempted to isolate and characterize the bacterial species from the clinical specimens using biochemical techniques. The enzyme, carbapenemase produced by the bacteria was isolated and determined by different assays. Another study identified the antibacterial, antioxidant and anticancer activities of Ganoderma lucidum by various chromatographic techniques. Anticancer activity was also assessed on HeLa cell lines using MTT assay and DPPH assay. In one of the chapters,the author discussed L-asparaginase, one of the widely exploited enzymes for the treatment of acute lymphoblastic leukemia (ALL). Also attempted to isolate and characterize the enzyme from soil samples collected from different locations at Kerala. The study indicated that soils can provide a rich source for L-asparaginase which has got ample application in pharmaceutical industries.

The studies on various geological aspects with respect to different geographical areas in Kerala soil has been included in the book. The vertical geochemical variation and elemental mobility of the lateritic terrain in the Makkaraparamba of Malappuram District, Kerala has been very well investigated. Under extremely oxidizing and leaching conditions, laterite

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soil transformed into a variety of rocks and further developed into stable secondary product in the existing humid tropical and subtropical environments. The hydrogeological conditions in Kumbala- Kaliyar river basin, Kasaragod district, Kerala was assessed by means of Vertical Electrical Sounding (VES). The digital spatial data output of the present study would be much helpful for planning and management of surface and sub-surface water resources of Kasaragod River basin in which the Kasaragod township is centrally located

The contributed chapters in the book written by the faculties of science stream in the light of the recent thinking and developments in the field of science and education.Science& Technology is now dominates almost every field of our activities. In summary , The faculties (Science stream) of GEMS Arts & Science college have made a n excellent attempt to bring about this book Homo Scientia".covering almost all the important areas from biological sciences to artificial intelligence. Every article has its own merits in both academic and research fronts..I record my grateful appreciation and thanks to the contributors of this book for their untiring efforts.

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ISOLATION AND PURIFICATION OF ANTI-CANCER ENZYME L-ASPARAGINASE FROM SOIL

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ABSTRACT

L-Asparaginase (L-asparagine aminohydrolase, E.C. 3.5.1.1) has been proven to be primary component in treating Acute Lymphoblastic Leukaemia (ALL), widely observed in pediatric and adult groups. At present, clinical formulations of L-Asparaginase are derived from bacterial sources such as *Escherichia coli* and *Erwinia chrysanthemi.* When these formulations administered to ALL patients which lead to several immunological and hypersensitive reactions. Nevertheless, the extracellular enzymes have an advantage over the intracellular ones; they could be produced plentifully in the culture medium under normal conditions and could be purified economically. Microbes isolated from soil samples are screened for the presence of L-asparaginase. The isolates were characterized through dialysis, ion exchange and gel filtration and identified the presence of L –asparaginase in soil samples collected from different locations. The production of L –asparaginase is carried out by submerged fermentation using Luria Bertani (LB) broth. The study revealed that the collected samples provide a rich source for L-asparaginase and can further utilized for

pharmaceutical purposes. Hence, there is a need for additional purification steps to remove toxicity induced by the involvement of enzymes such as glutaminase and urease.

INTRODUCTION

L-asparaginase belongs to a group of homologous amidohydrolases family that catalyzes the hydrolysis of amino acid L-asparagine to L-aspartate and ammonia. One of the major methods to treat most common type of childhood cancer Acute Lymphoblastic Leukaemia (ALL) is through the enzyme L-asparaginase. L-asparaginase aminohydrolase is a vital enzyme act as a chemotherapeutic agent and also possesses a wide range of medicinal properties. Due to their antimicrobial property, they are widely used for the treatment of infectious/autoimmune diseases not only in human but also for the treatment of canine and feline cancer. Their richest sources are bacteria, fungus and plants and owing to their wide range of medicinal properties, they had a greater demand for mass production. This enzyme had much significance for the treatment of leukaemia particularly ALL and are widely used to reduce the formation of carcinogenic acrylamides in baking and food industries.

Currently, clinical L-Asparaginase formulations are derived from bacterial sources such as *Escherichia coli* and *Erwinia chrysanthemi*. Microbial strains such as *Pseudomonas aeruginosa, Streptomyces gulbargensis, Aspergillus terreus, Aspergillus niger, Penicillium brevicompactum, Cladosporium* are the main source of L-asparaginase. Bacterial L-asparaginase cause hypersensitivity leading to allergic reactions and anaphylaxis. Three types of asparaginase have been reported; asparaginase made from Escherichia coli (also known by its brand Spectrila), from *Erwinia chrysanthemia* (Erwinase) and long acting (pegylated) asparaginase (also known as pegaspargase).

Recent studies on L-asparaginase have also been shown to have potential for preventing metastasis from solid tumor. Following, the most recent advances in protein engineering applied to the development of bio better ASNase (i.e., with reduced glutaminase activity, proteolysis resistant

and less immunogenic) using techniques such as site-directed mutagenesis, molecular dynamics, PEGylation, and bioconjugation.

Apart from medicinal uses, L-Asparaginase is also used in food industry to prevent acrylamide formation at high temperatures, which otherwise has carcinogenic and neurotoxic implications. The current study envisages attempting the isolation of L. asparaginase from soil samples collected from different locations.

METHODOLOGY

Sampling

Soil samples was collected from six different locations and diluted in sterile distilled water and inoculated on M9 medium supplemented with phenol red as indicator (Table 1).

Isolation and characterization of L. asparaginase

The inoculated agar plates were incubated at 37^{0}C for 24 hrs. The colonies picked from the plates and streaked on LB agar (Table 2) and incubated at 37^{0}C overnight. The culture was isolated and characterization was done through gram staining methods and biochemical tests.

Table 1: Composition of Minimal Salt Media (50 ml)

1	Dipotassium hydrogen orthophosphate	0.15 g
2	L- asparagine	0.5 g
3	Dihydrogen orthophosphate	0.3 g
4	Sodium chloride	025 g
5	Ammonium chloride	0.1 g
6	Magnesium sulfate	0.005 g
7	Agar	1.1 g
8	peptone	(pinch)
9	Distilled water	50 ml
10	Phenol red	0.018 g (pinch)

Table 2. Luria Bertani (LB) media Composition

1	Tryptophan	1 g
2	Sodium chloride	1 g
3	Yeast extract	0.6 g
4	Agar	2.1 g
5	Distilled water	100

Production and purification of L-Asparaginase

The production was carried out by submerged fermentation in which loop-full of bacterial culture was inoculated in a defined media (Table 3) and incubated at 37^{0}C in a rotary shaker for 48 hours. The enzyme produced were purified by means of salt precipitation, dialysis, ion exchange and gel filtration methods. The broth was centrifuged at 6000 rpm for 12 minutes. The supernatant was collected and ammonium sulfate was added. The precipitate was collected after centrifugation at 8600 rpm for 15 minutes. The samples were taken for dialysis and dialyzed fraction was poured into a burette over the ion exchanger (resins). The purification of the ample was done by gel filtration using potassium phosphate buffer at neutral pH.

Table 3. Production media

1.	Dipotassium hydrogen orthophosphate	0.15 g
2.	L- asparagine	0.5 g
3.	Disodium hydrogen phosphate	0.3 g
4.	Sodium chloride	0.25 g
5.	Ammonium chloride	0.1 g
6.	Magnesium sulfate	0.05

Biochemical assay

The enzyme assay was performed using Nessler's reagent by spectrophotometry for the estimation of ammonia produced L-asparaginase catalysis. The reaction mixture consists of L-asparagine (189mM), tris HCL (50mM) with pH 8.6 and are incubate for 10 minutes at room temperature. The reaction was stopped by the addition of 1.5 M TCA solution.The amount of protein was estimated by Lowry's method using bovine serum albumin as standard using 280 methods.

Enzyme Characterization

Effect of pH and temperature

The partially purified enzyme was incubated at pH (5 to 10). The reagents used were potassium phosphate buffer (0.1M, pH 7-8), sodium acetate (0.1M, pH 5-6) and glycine-NaOH (0.1 M, pH 9-10) and Tris HCl (pH-8).The optimum temperature for L-asparaginase activity was determined by incubating samples at 25^0, 30^0, 35^0, 40^0, 45^0, and 50^{0}C respectively for 30 minutes in a beaker.

Effect of substrate concentration and incubation time

The optimum activity of enzyme was evaluated at different asparagine concentration. The influence of incubation time was tested at 10-minute time intervals. The enzyme samples and are incubated at 37^{0}C for one hour. Three standards (standard 1, standard2 and standard3) was incubated for 30 minutes while six samples (S1, S2, S3, S4, S5, and S6) was incubated for 10, 20, 30, 40, 50, and 60 respectively at 10minutes interval.

Determination of molecular weight

The molecular weight of the enzyme was determined by using SDS PAGE with acrylamide gel and stacking gel containing 0.1% SDS. The gel was stained by using Coomassie brilliant blue and destaining was done using methanol, acetic acid, and distilled water.

RESULTS AND DISCUSSION

Isolation and identification of L-Asparaginase

Among the identified 34 organisms, 12 organisms was selected randomly and screened using minimal salt media. After incubation, pink and orange colored colonies was found. The activity of L-asparaginase was identified based on the pink color bacterial colonies. The colonies with pink zones around the colony were considered as L- asparaginase producing strains.

Production and purification

The pink color colonies produced more enzyme in sample (S2) at(OD = 4.640). The enzyme produced were purified and fine product was produced through dialysis, ion exchange and gel filtration.

Enzyme Characterization

Different factors such as incubation time, pH, temperature, and substrate concentration had a greater influence in enzyme activity. Enzyme activity is maximum at incubation for 10 minutes. The enzyme activity slowly declines when the incubation periods increased (Figure 1). The enzyme activity is maximum shown at acidic pH (3-4) (Figure 2). Maximum enzyme activity was showed at the temperature (30^{0}C to 40^0 C) (Figure 3). Maximum enzyme activity showed at the substrate concentration of sample (4.5 ml) (Figure 4).

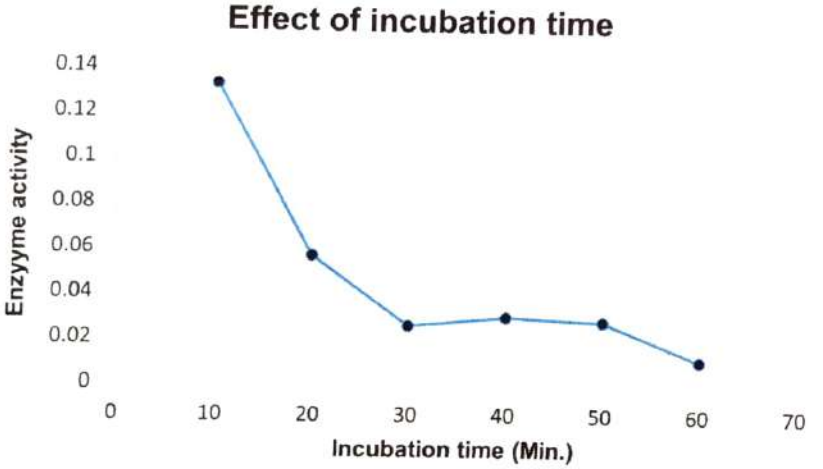

Fig 1. Effect of incubation time on enzyme activity

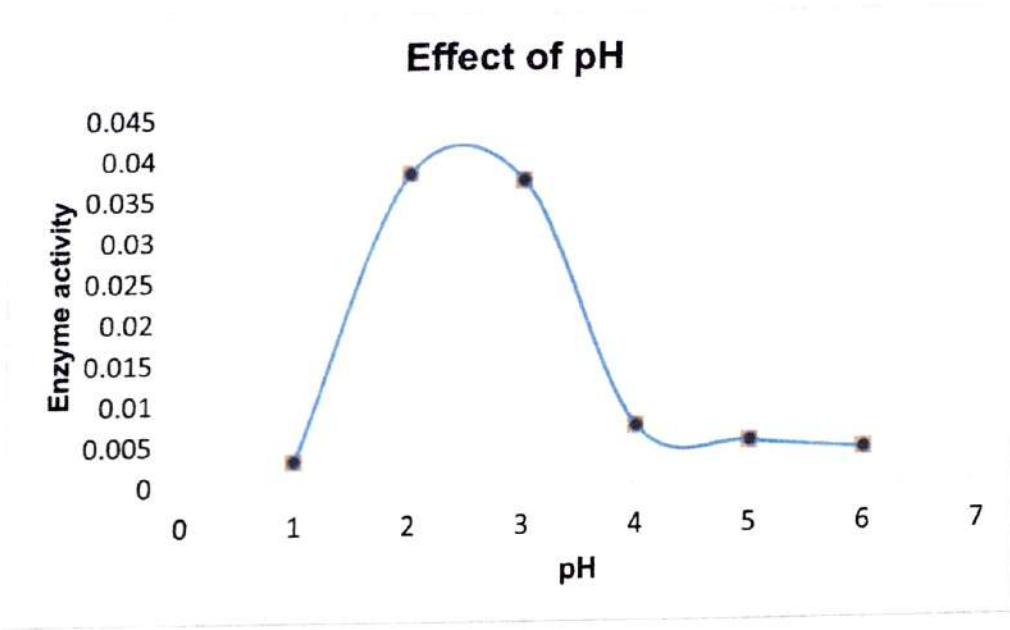

Fig 2. Effect of pH on enzyme activity

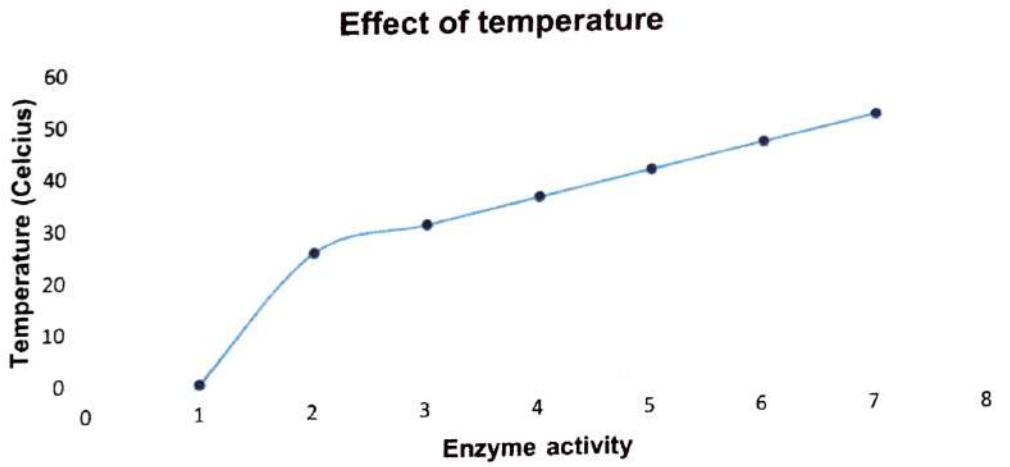

Fig 3. Effect of temperature on enzyme activity

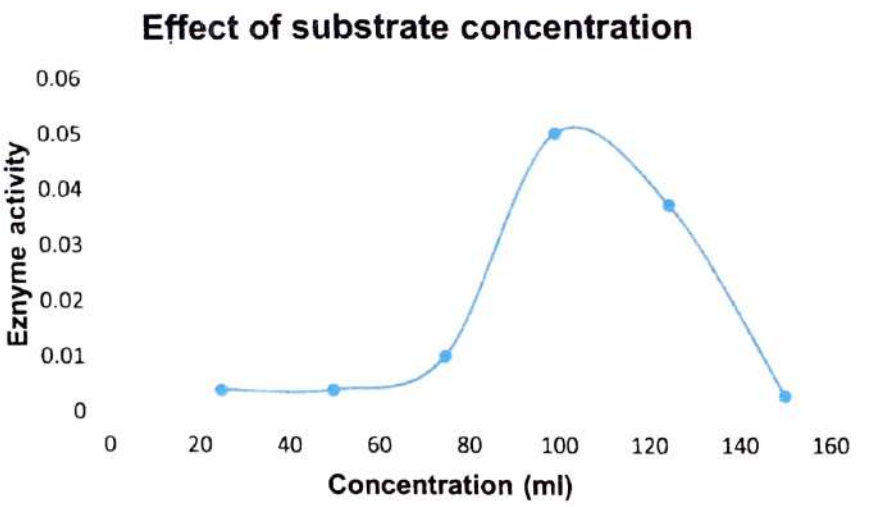

Fig 4. Effect of substrate concentration on enzyme activity

Molecular weight determination

A well-defined precise band were found in the gel resolved in poly acrylamide and after destaining method.

L-Asparaginase has received considerable attention as a primary component in the treatment of acute lymphoblastic leukemia (ALL). Extracellular enzymes have an advantage over the intracellular ones; they could be produced plentifully in the culture medium under normal conditions and could be purified economically. In this study the extracellular activity was more than the intracellular one, this offers easy enzyme recovery without the need for cell lysis.

The bacterial strain was isolated from soil samples collected from Azyme Biosciences, Bangalore. Strains producing L-asparaginase were identified by a pink colored colony on modified M9 agar medium with phenol red as an indicator for detection of L -asparaginase producing colonies. The microbial strain with the pink colored colony was selected for further studies. Previous studies, revealed the isolation of L-asparaginase was carried out by exploring different soil microorganisms like bacteria and fungi. The media used for the isolation for

actinomycetes from soil are on starch-nitrate agar. Six different soil samples of Vishakhapatnam regions were screened for potential producers of L-asparaginase using modified Czapek Dox's agar containing L-asparagine and phenol red indicator with pH 6.8. The strain isolated from slaughter house soil sample showed the maximum activity.

Purification of L-asparaginase was achieved by using ammonium sulfate saturation, Sephadex G-100 gel filtration, and CM-Sephadex C50 cation exchange column, DEAE sepharose CL-6B column. L-asparaginase activity was determined by hydrolysis of L-asparagine to release the ammonia which was measured by using Nessler's reaction. A mixture of 0.1 ml enzyme extract, 0.2 ml of 0.05M Tris-HCl buffer (pH 8.6) and 1.7 ml of 0.01M L-asparagine 21 Isolation and survey of L-asparaginase producing bacteria from soil was incubated for 10 min at 37°C. The reaction was stopped by the addition of 0.5 ml of 1.5 M trichloroacetic acid (TCA), and then centrifugation at 1000 rpm, 0.5 ml of supernatant was diluted to 7ml with distilled water and treated with 1 ml of Nessler's reagent. The color reaction was allowed to develop for 10 min and absorbance read at 480 nm with a spectrophotometer. Protein estimation was performed to determine total protein contents were estimated according to Bradford method with BSA as standard and the activity of L- asparaginase was assessed by the method of Mashburn and Wriston assay at 480 nm.

The results revealed that L-asparaginase activity was observed at 400C, pH 6 to 7 and 125ml asparagine concentration. The optimum alkaline pH of the enzyme is attributed to that the aspartate liberated by asparagine hydrolysis has lower affinity to the active catalytic site of the enzyme. This enables more binding of asparagine to the enzyme. On the other hand, at acidic pH the breakdown of asparagine by the enzyme results in the production of aspartic acid which has high affinity to the enzyme catalytic site, disabling the binding of asparagine to the enzyme. The same results were recorded by other researchers who found the maximum enzyme activity from *Streptomyces halstedii* and *Penicillium brevicompactum* at pH 8.0, while others reported the maximum activity from B. *licheniformis* and *Streptomyces gulbargensis* at pH 9.0. The molecular

weight of L-asparaginase was found to be varied according to the source of enzyme having molecular weight of 80kDa in *Corynebacterium glutamicum* and protein weight of 140kDa in *Streptomyces* sps. while *E.coli* possess smaller molecular weight ranges from 33-34kDa. The molecular weight of the extracted enzyme was determined by performing SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) according to the method of Laemmli with a separating acrylamide gel and stacking gel containing SDS. After the electrophoresis, the gel was stained with Coomassie brilliant blue R-250 and destained solution. SDS–PAGE of the enzyme preparation revealed only a single distinctive protein band for the pure preparation of L-asparaginase with an apparent molecular weight of ~53 kDa. Other reports on production and purification of L-asparaginase from E. coli revealed that the molecular weight was determined as 153 KDa with the help of SDS-PAGE. SDS-PAGE separation showed only one band characteristic of the enzyme preparation and no detectable contamination. Therefore, L-asparaginase was a homogeneous protein. The molecular weight of each band was determined at a molecular weight of 65 kDa. The molecular weight of L-asparaginase has been found to vary with the enzyme source of purified.

CONCLUSION

L-asparaginase is an extracellular enzyme which is a prime therapeutic agent for the treatment of Acute Lymphoblastic Leukemia (ALL). L asparagine is essential for the survival of both normal and cancer cells. Most normal tissue can synthesize L-asparagine for their metabolic needs while malignant cells cannot and must constantly rely on external supply. The results revealed that L- asparaginase activity was observed maximum at 40^{0} C, pH 6 to 7 in 125 ml asparagine concentration. The optimum alkaline pH of the enzyme is attributed to that the aspartate liberated by asparagine hydrolysis has lower affinity to the active catalytic site of the enzyme enable more binding. Studies reported the antioxidant and antitumor activity of the enzyme which can further used for various medicinal purposes.